

## Mechanisms of inflammatory pain

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One of the cardinal features of inflammatory states is that normally innocuous stimuli produce pain. Since the publication of the Melzack–Wall gate control theory in 1965,<sup>45</sup> it has been widely appreciated that the nervous system exhibits a range of responses according to different conditions ('neural plasticity'). Subsequent research has characterized the mechanisms by which these changes occur and highlighted the importance of environmental factors on perception of pain.

This review focuses on key peripheral mechanisms that result in the hypersensitivity state that accompanies inflammation. Recent studies are described which characterize a series of receptors, ion channels and transmitters involved in inflammatory pain. The mechanisms by which inflammatory mediators interact with neurones to produce hypersensitivity are also explored.

### The process of pain

In clinical settings it may be useful to identify several broad processes as being associated with pain: nociception, pain perception and a number of secondary consequences including suffering and pain behaviour.<sup>38</sup> Under this schema, nociception may be defined as the detection of noxious stimuli and the subsequent transmission of encoded information to the brain. In contrast, pain is essentially a perceptual process that arises in response to such activity (see Figure 1).

Symptoms and signs arising from normal tissues exposed to high intensity stimuli generally reflect the intensity, localization and timing of the initiating stimuli. In contrast, pain arising from inflamed or injured tissues may arise spontaneously in the absence of an external trigger. Alternatively, responses to noxious stimuli may be enhanced (hyperalgesia) or normally innocuous stimuli may produce pain (allodynia). These features are not specific and

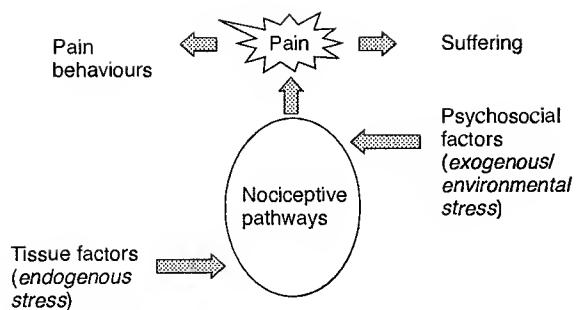
do not, in themselves, allow recognition of distinct pathophysiological mechanisms. The movement-related symptoms of osteoarthritis and the touch-evoked pain of herpetic neuralgia are both examples of mechanical allodynia although they clearly arise from different mechanisms. Given limitations with present terminology, the word hyperalgesia will be adopted throughout this review to describe the state of pain hypersensitivity that accompanies inflammation.

### Nociception

Cutaneous and deep somatic tissues are innervated by primary afferent neurones that synapse with second-order neurones in the dorsal horn of the spinal cord. Primary afferent neurones have three functions with respect to their role in nociception: detection of noxious or damaging stimuli (transduction); passage of the resulting sensory input from peripheral terminals to the spinal cord (conduction); and synaptic transfer of this input to neurones within specific laminae of the dorsal horn (transmission). Sensory information arising from noxious stimuli is then relayed to supraspinal structures including the thalamus and the brainstem. Powerful internal controls are present at all levels, as exemplified by descending modulatory systems.

### Transduction

The properties of receptors that detect either normal low-intensity stimuli or intense noxious stimuli differ in many important respects. Receptors for non-painful stimuli (such as light touch or movement) are characterized by specificity for a particular stimulus, a high degree of gain to amplify weak signals and rapid adaptation to increasing signal intensities.<sup>19</sup> In contrast, specificity is not so important after a noxious stimulus where the primary imperative is to



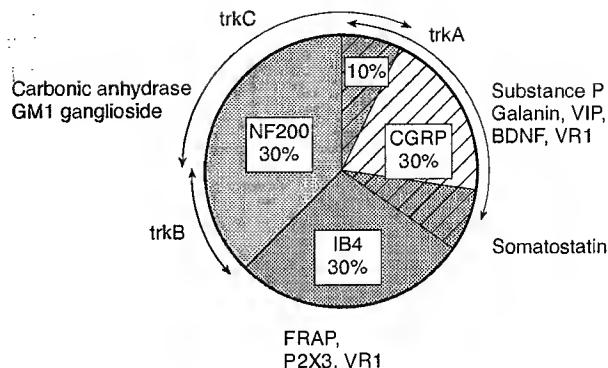
**Fig 1** Processes of pain. Activity in nociceptive pathways leads to the experience of pain. Such activity can be stimulated or modified by both endogenous and exogenous stress. Endogenous stressors include damage and/or inflammation within both neuronal and non-neuronal tissues whereas exogenous stress may be produced by psychosocial factors. The resultant pain behaviours and suffering can be measured and provide useful parameters for clinical assessment.

protect and remove the affected area as quickly as possible. Most high-threshold receptors therefore respond to a variety of thermal, chemical and mechanical stimuli and are defined as polymodal nociceptors. A further and clinically relevant characteristic is that far from adapting to an ongoing stimulus, the threshold for activation of nociceptors may in fact fall such that relatively trivial stimuli now produce pain. This process of 'sensitization' will be discussed in later sections.

### Conduction

Within adult dorsal root ganglia (DRG), large diameter cells have high levels of neurofilament and give rise to myelinated  $A\beta$  fibres and a proportion of more thinly myelinated  $A\delta$  fibres. Representing about 40% of lumbar DRG cells, they express trkB and trkC, which are high-affinity tyrosine kinase receptors for brain-derived neurotrophic factor (BDNF) and neurotrophin-3 (NT-3), respectively.<sup>47</sup> In contrast, small diameter cells give rise to mainly unmyelinated axons. They can be differentiated histochemically into two distinct populations including those cells which constitutively synthesize neuropeptides and those which bind the lectin IB4 (Fig. 2).

There is an extensive overlap (around 92%) between small cells expressing neuropeptides and the high-affinity receptor for nerve growth factor (NGF), trkA.<sup>4</sup> These cells, which are at least partially regulated by NGF, project to areas associated with nociceptive transmission and may be involved in neuromodulation and peripheral neurogenic inflammation.<sup>63</sup> The IB4 population of cells express trkA and respond to NGF in development, but trkA expression is down-regulated in the early postnatal period.<sup>6</sup> These cells express the receptor for tyrosine kinase, c-ret, and are regulated by glial cell line-derived neurotrophic factor (GDNF).<sup>8</sup> Although their exact function remains unclear,



**Fig 2** Pie chart summarizing the three main populations of DRG cells observed in rodents and their expression of neurotrophin receptors. Large diameter cells give rise to myelinated axons and have high levels of neurofilament (NF200). Small cells, with mainly unmyelinated axons, comprise the remaining two populations. One population of small cells constitutively synthesizes neuropeptides and responds to the growth factor, NGF, whereas the other expresses the lectin IB4 and responds to GDNF. Both small cell populations express the VR-1 receptor and are thought to be nociceptors (figure kindly provided by J. V. Priestley).

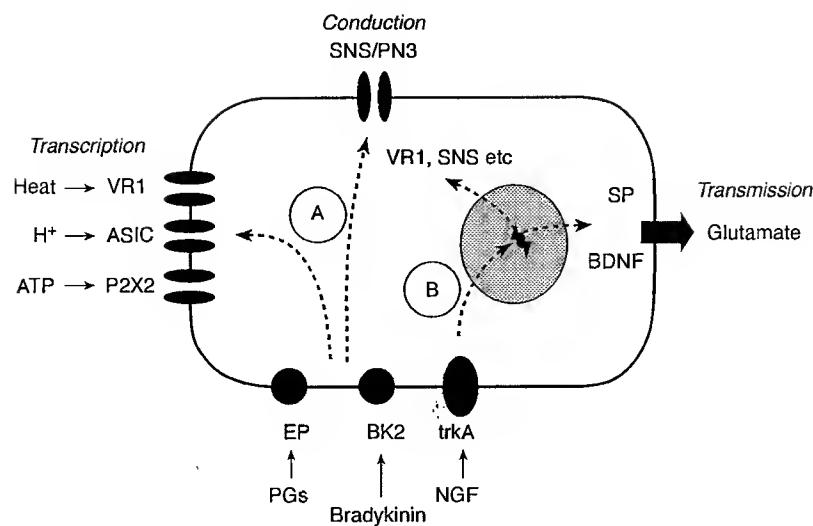
many of these cells express vanilloid receptor-1 (VR-1) and are thought to be nociceptors.

Neurophysiological studies confirm that, under normal conditions, rapidly conducting  $A\beta$  fibres (with conduction velocities  $>30\text{ m s}^{-1}$ ) are mainly concerned with non-noxious input from specialized encapsulated receptors. In contrast, most small diameter fibres, including  $A\delta$  fibres (with conduction velocities of  $2.5\text{--}30\text{ m s}^{-1}$ ) and C fibres (conduction velocities  $<2.5\text{ m s}^{-1}$ ), have free nerve endings and respond to noxious stimuli (Fig. 3).<sup>30</sup> Whilst most C fibres show polymodal responses, some are exclusively chemosensitive under normal conditions and do not respond to mechanical and thermal stimuli. These silent or 'sleeping' nociceptors were first described in joints but were later found in other tissues.<sup>60</sup>

### Transmission

In the first instance, spinal responses to non-tissue-damaging noxious stimuli are mediated by the excitatory amino acid, glutamate, acting on  $\alpha$ -amino-3-hydroxy-5-methylisoxazole (AMPA) receptors. Importantly, repetitive stimulation or greater stimulus intensities, such as those associated with tissue damage, are associated with the functional expression of a second glutamate-responsive receptor, the *N*-methyl-D-aspartate (NMDA) receptor.<sup>25</sup> Activation of this receptor produces a sequence of events leading to increased excitability of dorsal horn neurones (Fig. 4).

Greater stimulus intensities are associated with the release of neuropeptides, including substance P, from central terminals of C fibres. Substance P, acting via



**Fig 3** Influences on primary afferent neurones leading to 'peripheral sensitization'. Under normal circumstances, high-intensity stimuli are encoded by specialized membrane-bound receptors. Conduction of message to central terminals and transmission to spinal neurones is mediated by ion channels and excitatory amino acids, respectively. (A) During the early stages of inflammation, mediators such as prostaglandins (PGs) and bradykinin (BK) change the sensitivity of receptors and reduce activation threshold for conducting ion-channels. (B) Longer-term changes include transcriptional events mediated by cytokines and growth factors resulting in enhanced production of receptors, ion channels and central transmitters/modulators (modified from reference 74).

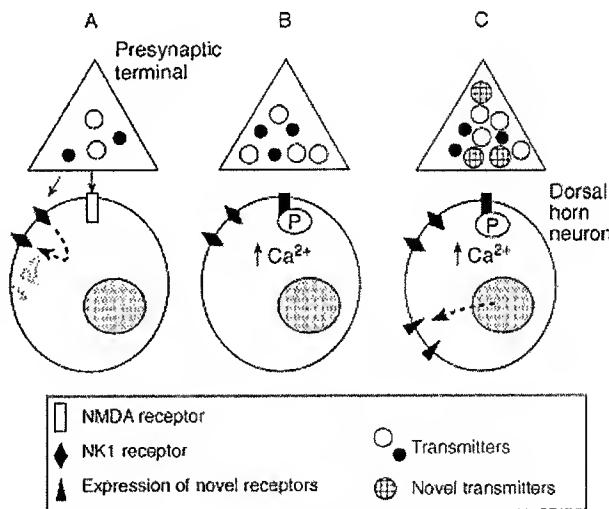
neurokinin (NK-1) receptors located on dorsal horn neurones, generates a greater post-synaptic response and enhances the activity of NMDA receptors.<sup>66</sup> This interaction takes place through the activation of protein kinase C, which phosphorylates the NMDA receptor, thereby changing its responsiveness to subsequent stimuli. Under normal circumstances, Mg<sup>2+</sup> binding blocks the NMDA receptor but the alteration in Mg<sup>2+</sup> binding kinetics allows release of Mg<sup>2+</sup> from the receptor and permits glutamate-induced activation and subsequent depolarization of the cell membrane.<sup>74</sup>

## Receptors

The detailed biochemical and cellular mechanisms underlying the detection of painful stimuli are being revealed as more molecules are cloned and their function is elucidated. Recently, a series of ion-channel-linked receptors related to sensory transduction of noxious stimuli has been described. These include heat-activated vanilloid receptors and others sensitive to protons and products of purine metabolism.

### Vanilloid receptors

Most nociceptors can be characterized by their sensitivity to capsaicin, the active ingredient in spicy 'hot' foods. One of the major advances in pain research over the past decade has been the isolation of a functional cDNA encoding the capsaicin receptor in sensory neurones.<sup>16</sup> VR-1 is a ligand-gated, non-selective cation channel. It belongs to a family of



**Fig 4** Development of 'central sensitization' in the spinal dorsal horn after inflammation of peripheral tissues. (A) Early phase with activation of C fibres: Glutamate (open spheres) and substance P (black spheres) are released from C fibres. Glutamate and substance P NK1 receptors are activated in dorsal horn neurones with the latter undergoing internalization and recirculation to the membrane. (B) Sensitization phase: Pre-synaptic up-regulation of neurotransmitter production together with ongoing activity in C fibres results in increased transmitter release with sustained influx of Ca<sup>2+</sup> ions into dorsal horn neurones. Phosphorylation of the NMDA receptor by activated kinases allows the NMDA receptor to operate at resting membrane potential level and further enhances the accumulation of intracellular Ca<sup>2+</sup>. (C) Established phase of chronic inflammatory pain. Synthesis of novel transmitters, growth factors and ion channels may lead to phenotypic changes within the nociceptive system (e.g. production of substance P in large fibres).

receptors that also includes the vanilloid receptor-like protein (VRL-1) and the stretch-inactivated channel (SIC). VR-1 is primarily distributed in small diameter afferent neurones although recently a more widespread expression in the central nervous system (CNS) has been described.<sup>46</sup>

In addition to being sensitive to capsaicin, VR-1 responds to moderate thermal stimuli (approximately 43°C), suggesting a heat-transduction role for this receptor. Interestingly, VRL-1 does not respond to capsaicin or moderate heat but is activated by high temperatures with a threshold of approximately 52°C.<sup>17</sup> Two recent studies<sup>18-22</sup> have demonstrated normal responses to acute noxious thermal stimuli in VR-1 knockout mice. However, hyperalgesic responses in a variety of inflammatory models were substantially attenuated or absent. Significantly, mechanical hyperalgesia was unaffected.

VR-1 responds to protons,<sup>49</sup> suggesting that its activity might be enhanced within the acidic environment of inflamed tissues. Other putative endogenous ligands include the cannabinoid receptor agonist anandamide<sup>78</sup> and the lipoxygenase product 12-(S)-hydroperoxy-eicosatetraenoic acid (12-(S)-HPETE).<sup>33</sup> Taken together, current evidence supports the conclusion that vanilloid receptors respond to multiple pain-producing stimuli, but whether VR-1 and related receptors play some form of integrative role following tissue injury remains unclear (for reviews see references 56 and 65).

#### *Acid-sensing receptors*

Recently, a new family of ion channels which are selectively activated by protons has been described.<sup>69</sup> These channels belong to the acid-sensing ion-channel (ASIC) group of receptors, which respond to low pH by producing a rapidly inactivating current in addition to a sustained sodium current.<sup>68</sup> They occur widely throughout the nervous system, with the ASIC-3 (DRASIC) subtype being most closely associated with dorsal root ganglion cells.<sup>68</sup> In addition to responding to acidic environments, it has been postulated that certain subtypes of ASIC receptors, together with stomatins, might also be involved in mechanosensitivity.<sup>70</sup>

#### *Purinergic receptors*

Adenosine and related phosphate derivatives (AMP, ADP and ATP) have been shown to produce pain in human subjects.<sup>13</sup> P2X purinoreceptors are ionotropic ligand-gated ion channels mediating fast synaptic transmission by extracellular ATP.<sup>14</sup> One receptor subtype, P2X3, is expressed selectively in small diameter neurones that label with the lectin IB4, suggesting that it plays a role in nociception. Responses to ATP are enhanced during inflammation in a number of experimental models; it has been suggested that sympathetic nerves, vascular endothe-

lial cells or epithelial cells were the source of endogenous ATP in these models.<sup>15</sup>

#### **Ion channels**

Conduction with the nervous system is mediated in the first instance by voltage-gated ion channels. Although ion channels have a ubiquitous distribution, recent studies have identified a number of channels that appear to have a more selective role in nociception.

#### *Sodium channels*

Sodium channels can be classified into those that are sensitive to the puffer fish toxin tetrodotoxin (TTX-S) and those that are resistant (TTX-R). Whereas large diameter neurones express only TTX-S sodium channels, small diameter nociceptor neurones express both TTX-S and TTX-R channels.<sup>29</sup> Two sensory neurone-specific TTX-R sodium channels have been cloned, termed SNS/PN3 and SNS2/NaN, respectively.<sup>1</sup> The SNS/PN3 channel is closely associated with the nociceptor population within DRG<sup>51</sup> and the amounts of SNS/PN3 protein are increased during chronic inflammation.

Consistent with a role for SNS/PN3 in inflammatory pain states, prostaglandin E2 (PGE2), adenosine and serotonin all enhance channel sensitivity<sup>27</sup> and intrathecal administration of SNS/PN3 antisense oligonucleotides reverses inflammation-induced hyperalgesia.<sup>55</sup> Local anaesthetics, such as lignocaine, and anticonvulsants, including carbamazepine and phenytoin, block sodium channels but side effects within the CNS and elsewhere limit their widespread clinical application. By selectively affecting generation of action potentials in nociceptive neurones, blockade of TTX-R channels presents an attractive and highly specific therapeutic strategy for relieving both neuropathic and chronic inflammatory pain states.

#### *Calcium channels*

A range of voltage-gated calcium channels have been identified as being involved in transmitter release and prolonged excitatory states of the neuronal membrane.<sup>67</sup> It is noteworthy that the anticonvulsant gabapentin and related structures have high affinity and specificity for the  $\alpha 2\delta$  subunit of these channels.<sup>28</sup> Gabapentin has found widespread acceptance in patients with diabetic and post-herpetic neuralgia,<sup>32</sup> but appears to be less effective in individuals with inflammatory pain. Similarly, blocking calcium channels using  $\omega$ -conotoxin, a toxin derived from snails of the genus *Conus*, produces analgesia<sup>76</sup> but the effect does not allow differentiation between the various channels and has a limited therapeutic window. Potentially, selective blockade of the pre-synaptic N-type channel, which controls transmitter release at the dorsal horn, provides a useful target for broad-spectrum analgesics.

## Plasticity

Plasticity may be regarded as the property of the nervous system that enables it to modify its function according to different conditions.<sup>21</sup> It is pivotal to the development of the hypersensitivity state that underlies inflammatory pain. Ongoing studies are revealing how pain hypersensitivity is the consequence of early post-translational changes, including phosphorylation of membrane-bound proteins, as well as later transcription-dependent changes in effector genes at multiple levels along the nociceptive pathway.

### *Peripheral sensitization*

Tissue injury results in the release of inflammatory mediators from damaged cells including ions ( $K^+$ ,  $H^+$ ), bradykinin, histamine, 5-hydroxytryptamine (5-HT), ATP and nitric oxide. Activation of the arachidonic acid pathway leads to the production of prostanoids and leukotrienes. Recruited immune cells release further mediators including cytokines and growth factors. Some of these mediators activate peripheral nociceptors directly and lead to spontaneous pain, whereas others act indirectly via inflammatory cells to stimulate the release of additional pain-inducing (algogenic) agents. Importantly, inflammatory mediators also act to modify the response properties of primary afferent neurones to subsequent stimuli (peripheral sensitization). This may arise as a result of changes to the sensitivity of receptor molecules themselves, or via modulation of voltage-gated ion channels.

### *Bradykinin*

Bradykinin is released on tissue injury and makes an important early contribution to the inflammatory cascade. When given experimentally to human subjects, it produces pain, inflammation and hyperalgesia.<sup>44</sup> Bradykinin and kallidin together with their degradation products des-Arg<sup>9</sup> bradykinin and des-Arg<sup>9</sup>-kallidin have complex effects on primary afferent neurones, including both activation and sensitization by direct and indirect pathways (for a review see reference 26).

Bradykinin B2 receptors, which bind bradykinin and kallidin, are constitutively and abundantly expressed on both neurones and non-neuronal cells. Consistent with these findings, the selective and high affinity B2 receptor antagonist, Bradyzide, blocks inflammatory hyperalgesia in animal models.<sup>12</sup> In contrast to bradykinin, des-Arg<sup>9</sup> bradykin selectively activates B1 receptors. Interestingly, B1 receptor agonists produce pain only during inflammation, suggesting that enhanced expression of the B1 receptor or sensitization of the receptor is required.<sup>26</sup>

### *Cytokines*

Cytokines play an important role in the initiation and maintenance of inflammatory diseases as mediators of cell-cell interactions. In addition to their enhancing and inhibitory effects on immune and inflammatory cells,

cytokines exert considerable influence over sensory neurones. Similar to other mediators, cytokines may act directly on nociceptors or, more commonly, indirectly, stimulating the release of agents such as prostaglandins. During acute phases, cytokines appear to induce sensitization via receptor-associated kinases and phosphorylation of ion channels whereas in chronic inflammation transcriptional up-regulation of receptors and secondary signalling become more important.<sup>52</sup>

Most studies to date have focused on the pro-inflammatory cytokines including tumor necrosis factor alpha (TNF $\alpha$ ), interleukin-1 (IL-1), IL-6 and the chemokine IL-8. Intradermal injections of these agents generally produce both mechanical and thermal hyperalgesia. Antibodies against TNF $\alpha$  reduce hyperalgesia in inflammatory models<sup>75</sup> and the use of novel anti-TNF therapies in rheumatoid arthritis is accompanied by substantial reductions in pain scores.<sup>42</sup> More modest reductions have been observed after anti-IL-1 therapy.<sup>11</sup> IL-6 knockout mice show reduced mechanical and thermal hyperalgesia in response to inflammatory stimuli<sup>77</sup> or after chronic nerve constriction.<sup>48</sup>

### *Prostaglandins*

Prostaglandins are important mediators of inflammation, fever and pain. They are synthesized by the constitutive enzyme, cyclo-oxygenase-1 (COX-1), and its isoform enzyme COX-2, which is induced in peripheral tissues by cytokines, growth factors and other inflammatory stimuli.<sup>5</sup> Although in some situations prostaglandins contribute to pain by directly activating nociceptors, they are generally considered to be sensitizing agents. Prostaglandins increase levels of cyclic AMP and may enhance nociceptor sensitization by reducing the activation threshold for TTX-R sodium channels via a protein kinase A pathway.<sup>27</sup> They sensitize primary afferent neurones to bradykinin and other mediators<sup>50</sup> and are likely to be involved at multiple sites along the nociceptive pathway.<sup>59</sup>

COX-1 and COX-2 have been identified in the brain and spinal cord of humans and rats and both appear to be constitutively expressed in these tissues.<sup>71</sup> Recent studies using selective COX knockouts have suggested that these enzymes might subserve different mechanistic pathways and are possibly gender specific.<sup>5</sup> Whereas COX-1-deficient mice show reduced nociceptive activity to a variety of noxious stimuli, less marked changes are observed in COX-2-deficient mice. Rather surprisingly, nociceptive activity is reduced only in models of slowly developing diffuse pain in female, but not male, COX-2-deficient mice. Furthermore, up-regulation of COX-1 has been observed in spinal tissues of COX-2-deficient mice whereas compensatory up-regulation of COX-2 has not been observed in COX-1-deficient animals.<sup>5</sup>

### *Growth factors*

Neurotrophic growth factors, including NGF, make significant and long-lasting contributions to the changes of neurone sensitivity observed during inflammation. NGF

mRNA and/or protein has been identified in various cell types including fibroblasts, keratinocytes, Schwann cells and a range of immune cells. A large number of inflammatory mediators act to increase NGF production, particularly IL-1 $\beta$  and TNF $\alpha$ .<sup>75</sup> Consistent with this, increased levels of NGF have been reported in animal models of inflammation and in human disorders including arthritis, cystitis and asthma.<sup>39</sup>

The importance of NGF in mediating inflammation-induced hyperalgesia has been highlighted by a number of studies showing very significant reductions in enhanced responses using a variety of anti-NGF strategies, including the use of novel sequestration antibodies (reviewed in reference 39). In human subjects, NGF produces cutaneous hyperalgesia at the injection site and widespread deep pain which persists for several days. It is probable these sensitizing effects are at least partially mediated by direct effects on nociceptors themselves and partially via mediators released by NGF-activated mast and other inflammatory cells. A role for sympathetic neurones has also been reported.

During the acute stages of an inflammatory response, neuronal trkA activation leads to tyrosine phosphorylation of intracellular targets including ion channels. Over the longer term, NGF exerts a more global influence by regulating the expression of the neuropeptides, substance P and calcium gene-related peptide (CGRP), as well as receptors including VR-1 and bradykinin B2, and ion channels such as SNS (reviewed in reference 35).

In addition to their classic trophic actions, neurotrophins can be synthesized by neurones and influence synaptic transmission. In particular, BDNF is synthesized by small DRG neurones, packaged in dense-cored vesicles, and transported within axons into terminals in the dorsal horn of the spinal cord.<sup>47</sup> BDNF has potent effects on spinal cord neurones and has been implicated in the central sensitization associated with inflammation.<sup>43</sup> Production of BDNF is increased by exogenous NGF<sup>47</sup> and by inflammation<sup>20</sup> and the increase in BDNF associated with inflammation can be reduced by treatment with antibodies to NGF.<sup>20</sup>

#### *Neurogenic factors*

The nervous system acts in concert with the immune and endocrine systems to constitute an interactive, communicative network. Neuropeptides such as substance P and CGRP are available for release from distal as well as central terminals of small diameter peptidergic neurones. They have a broad spectrum of effects within peripheral tissues and make a significant contribution to the so-called 'wheal and flare response' that follows cutaneous injury. In these circumstances, substance P is believed to act primarily on post-capillary venules to produce plasma extravasation, whereas CGRP acts on arterioles to produce vasodilation. A synergistic interaction between these peptides has been observed.<sup>10</sup>

It is probable that neuropeptides released from peripheral terminals make largely indirect contributions to nociceptor activity during inflammation. Although a detailed description of the pro-inflammatory effects of neuropeptides is beyond the scope of this review, most research to date has centred on substance P and related tachykinins (for a review see reference<sup>40</sup>). Substance P degranulates mast cells to produce histamine release, induces release of PGE2 and collagenase from synoviocytes and may stimulate the release of cytokines from macrophages, although this remains controversial.<sup>37</sup> Substance P has also been shown to have chemotactic properties with respect to T cells, monocytes, neutrophils and eosinophils.<sup>31</sup>

#### *Inhibition of peripheral sensitization*

##### *Non-steroidal anti-inflammatory drugs*

Non-steroidal anti-inflammatory drugs (NSAIDs) act to inhibit COX enzymes and reduce the formation of prostaglandins. Whilst the non-selective inhibition of COX produces a significant antihyperalgesic effect and emphasizes the importance of prostaglandins in inflammatory hyperalgesia, clinical use is limited by serious gastrointestinal side effects (for a review see reference 53). The recent introduction of selective COX-2 inhibitors provides a potential means to reduce these effects. The analgesic efficacy of selective COX-2 inhibitors in rheumatoid arthritis appears similar to that of non-selective inhibitors,<sup>62</sup> although long-term studies are awaited.

To circumvent problems associated with COX, the actions of prostaglandins can be substantially reduced by selective receptor blockade. The most promising approach uses antagonists of the EP receptor subfamily, which are present on sensory neurones and are activated by PGE2. IP receptors may also contribute to the development of inflammatory hyperalgesia, once activated by the inflammatory prostanoid PGI2. There is extensive experimental evidence for the pro-inflammatory effects of both PGE2 and PGI2 in the joint<sup>34,61</sup> and selective blockade of EP/IP receptors provides an effective antihyperalgesic strategy in animal models.<sup>9</sup>

A further alternative is offered by nitric oxide-releasing derivatives of NSAIDs.<sup>24</sup> Development of so-called nitro-aspirin and various combination of NSAIDs with nitric oxide allows greater anti-nociceptive and anti-inflammatory effects in inflammatory models of pain compared with the parent NSAID without damage in the gastrointestinal tract.<sup>2</sup>

#### *Opiates*

Opiates are produced by immune cells, and opioid receptors are present in peripheral tissues.<sup>64</sup> Expression of  $\mu$ ,  $\delta$  and  $\kappa$  receptors increases in primary afferent neurones during inflammation and selective agonists block spontaneous firing of fibres which innervate inflamed skin.<sup>3</sup> Opioid agonists developed for peripheral use (e.g. loperamide) show antinociceptive activity in inflammatory conditions

such as experimental arthritis.<sup>23</sup> Potentially, peripherally acting opioid compounds may provide pain relief in inflammatory conditions by systemic or topical application.

#### Cannabinoids

A novel approach to inhibiting peripheral sensitization is provided by cannabinoids. Topical application of cannabinoid receptor agonists blocks nociception in inflammatory models of pain.<sup>34</sup> Consistent with this finding, the natural endogenous ligand of cannabinoid receptors, anandamide, when given systemically, exerts analgesia. There are two types of cannabinoid receptor, CB1 and CB2. The former is expressed on central and peripheral neurones as well as on non-neuronal cells, whereas the latter is of non-neuronal origin and is present on immune cells. Activation of the CB1 receptor is negatively coupled to adenylyl cyclase and blocks excitability and activation of primary afferents.<sup>58</sup> Activation of the CB2 receptor may produce antinociceptive effects via inhibition of immune cell functions (for a review see reference 57).

In addition to central activity in pain pathways,<sup>36</sup> the strong peripheral presence of CB1 receptors in primary afferent neurones offers an alternative site for analgesic intervention. Although there is no doubt about the central antinociceptive effects of cannabinoids on their own and in co-operation with the opioid system,<sup>57</sup> the preferred route is the development of peripherally acting CB1 receptor antagonists, thereby prohibiting central side effects.

#### Central sensitization

Whilst pain hypersensitivity after an inflammatory stimulus is contingent to a large degree on peripheral sensitization, other mechanisms are also involved. Sustained or repetitive activation of primary afferent fibres produces substantial changes to the function and activity of central neurogenic pathways. In addition to glutamate, which dominates communication between the periphery and the spinal cord, neuropeptides such as substance P and neurotrophic factors such as BDNF are released from central terminals of primary afferents during inflammatory conditions. They serve to act as co-transmitters and induce long-lasting changes in spinal excitability known collectively as 'central sensitization' (Fig. 4).<sup>73</sup>

Increased release of peptide transmitters from primary afferent fibres activates second messenger systems and results in increased influx of  $\text{Ca}^{2+}$  ions and phosphorylation of proteins. During prolonged inflammation, activation of kinases produces transcriptional changes (for review see reference 74). The net result is that the responsiveness of dorsal horn cells, both to existing inputs and to previously sub-threshold inputs, is increased, producing: (i) exaggerated responses to normal stimuli; (ii) expansion of receptive field size; and (iii) reduction in the threshold for activation by novel inputs (e.g. from mechanoceptive A fibres).

The most plausible theory for central sensitization suggests that the NMDA receptor occupies a central position in this phenomenon. NMDA receptor antagonists are antinociceptive, but the therapeutic applicability of present antagonists is limited by the ubiquitous expression of the receptor. A number of endogenous mediators, including prostaglandins, nitric oxide, opioids and adrenergic agonists, also influence the excitability of spinal neurones. Whereas prostaglandins and nitric oxide appear to facilitate spinal excitability,  $\alpha$ 2 adrenergic and opioid receptor agonists produce analgesia by presynaptic inhibition of C-fibre neurotransmitter release and post-synaptic hyperpolarization of second-order neurones.<sup>8</sup> Co-administration of intrathecal morphine and selected  $\alpha$ 2 agonists or NSAIDs results in substantial analgesic synergy<sup>41</sup> and highlights a role for combination therapy in clinical settings.

#### Conclusions

The complex mechanisms underlying the modulation of mechanical, thermal and chemical transduction have started to emerge through the characterization of receptors, ion channels and neurotransmitter/modulator proteins. Changes in the sensitivity of nociceptive neurones underlie development of the tissue hypersensitivity associated with inflammation.

Recognition of the necessity for new strategies for the management of pain has led to the development of innovative drugs with favourable side-effect profiles. The introduction of ion channel blockers and selective inhibitors of COX-2 provides two obvious examples. Looking ahead, the characterization of specific pathophysiological changes underlying particular inflammatory diseases is set to produce a qualitative change in pain management and signals, for the first time, the possibility of diagnosis-based analgesic medication.

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